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New record of the mangrove rivulid *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Cynolebiidae) in the Pará state, northern Brazil

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Abstract: The mangrove killifish *Kryptolebias hermaphroditus* is reported to the southeast and northeast regions of Brazil. Recently, a specimen of *K. hermaphroditus* was collected in a shallow running seawater stream at Ajuruteua beach, Pará state, northern Brazil. This new record is ca. 1,350 km from the nearest previously known occurrence in Rio Grande do Norte state, Brazil. Morphological, molecular, and ecological aspects of this species are described.

Key words: estuarine fish; intertidal habitat; mitochondrial DNA; Neotropical Region; South America.

Three species of *Kryptolebias* Costa, 2004 (family Cynolebiidae) are known to occur in fresh and brackish estuarine waters along the western Atlantic coast. *Kryptolebias marmoratus* (Poey, 1880) was documented in coastal areas of Florida and the Caribbean, including the Bahamas and Belize. On the other hand, *Kryptolebias hermaphroditus* Costa, 2011 is occurs along the south coast of the Western Atlantic; it is endemic to southeastern Brazil (Rio de Janeiro, São Paulo, and Espírito Santo states) and is sympatric with *K. ocellatus* Hensel, 1868 (Taylor 2003; Tatarenkov et al. 2009; Tatarenkov et al. 2011; Sarmento-Soares et al. 2014). However, *K. hermaphroditus* had been recorded from Rio Grande do Norte (Lira et al. 2015), and this occurrence was believed to be the northernmost record of this species until now (Figure 1: localities 11 and 12).

These three cynolebiid species are commonly called "mangrove killifishes", and are the only ones living in brackish waters, because most other cynolebiids occur in freshwater systems (Costa et al. 2010). Moreover, the mangrove killifishes is the only known hermaphroditic vertebrate able to self-fertilize (Tatarenkov et al. 2009; Costa et al. 2010).

Here, we present a new record of *K. hermaphroditus* from northern Brazil, Pará state. This record is ca. 1,350 km northwest of the closest previously known occurrence.

A single individual of *K. hermaphroditus* was collected in December 2014 at Ajuruteua beach. This site is near the city of Bragança, Pará, Brazil (00°51′05.69″ S, 046°36′00.14″ W; Figures 1, 2). The specimen was collected under permit #121122 issued by ICMBio/SISBIO – IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis).

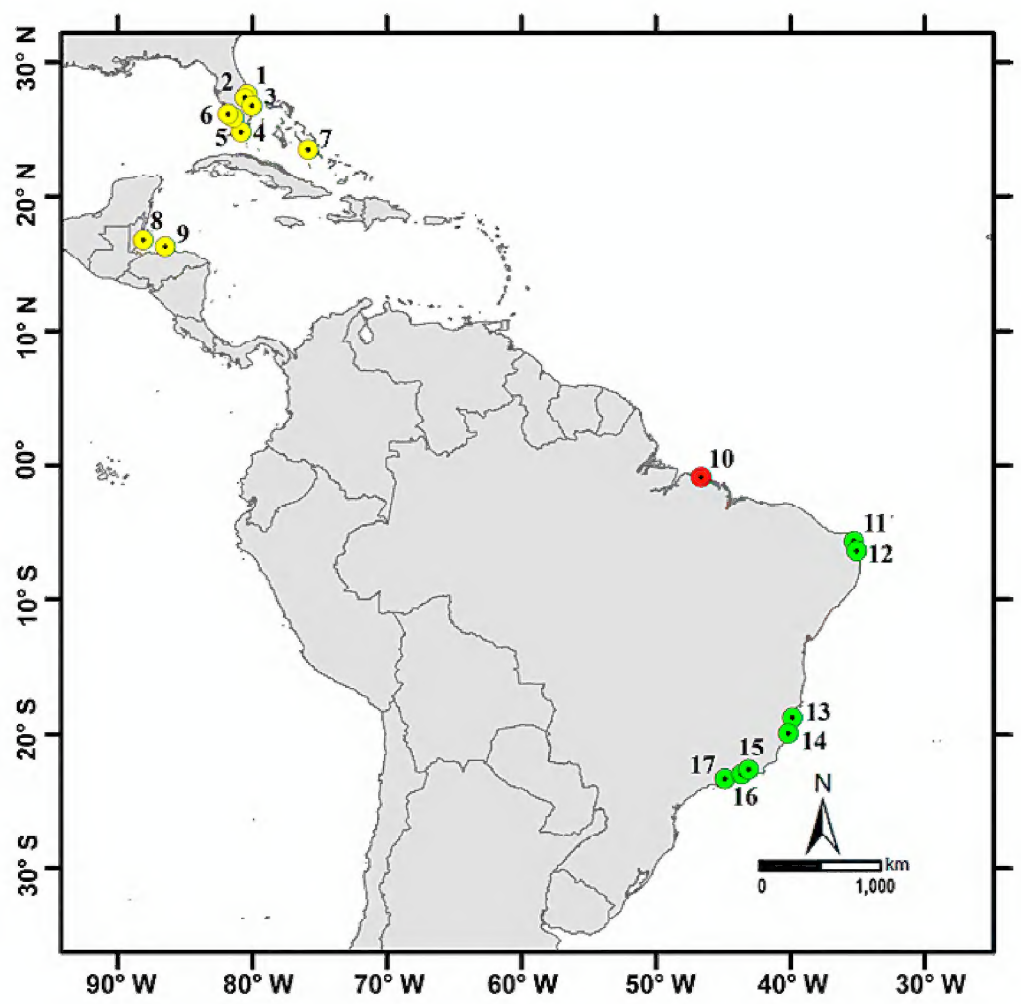

Figure 1. Yellow circles indicate previously recorded localities for *K. marmoratus*: 1) Vero Beach, Florida; 2) St. Lucie County, Florida; 3) West Palm Beach, Florida; 4) Long Key, Florida; 5) Everglades, Florida; 6) Naples, Florida; 7) Exumas, Bahamas; 8) Twin Cays, Belize; 9) Utila, Bay Island, Honduras. Red circle (10) indicates the new locality of *K. hermaphroditus* at Ajuruteua beach, Pará, northern Brazil. Green circles indicate previously recorded localities for *K. hermaphroditus* and *K. ocellatus* (northeastern and southeastern Brazil): 11) Ceará-Mirim River, Rio Grande do Norte; 12) Curimataú River, Rio Grande do Norte; 13) São Mateus River, Espírito Santo; 14) Preto do Sul River, Espírito Santo; 15) Iriri River, Rio de Janeiro; 16) Fazenda Beach, Parque Estadual da Serra do Mar, São Paulo; and 17) Piracão River, São Paulo.

Figure 2. *Kryptolebias hermaphoditus*, 34.2 mm SL (catalog number: GEA.ICT 02458) from Ajuruteua beach, Pará state, northern Brazil.

The specimen (Figure 2) was captured in a shallow running stream of clear water with a salinity of 38 and located in the intertidal zone at Ajuruteau beach, which forms during the low tide (Figure 3). The stream was surrounded by *Laguncularia racemosa* vegetation. The time capture was approximately at 16:00 h, 12 December 2014, in the end of the full moon cycle. *Poecilia vivipara* Bloch & Schneider, 1801, *Bathygobius soporator* (Valenciennes, 1837), *Microgobius meeki* (Evermann & Marsh, 1899), *Mugil* sp., and *Eleotris pisonis* (Gmelin, 1789) were also collected from the samestream as *K. hermaphroditus*.

The specimen of *K. hermaphroditus* was initially preserved in 100% ethanol and a sample of muscle tissue was removed and transfered to 90% ethanol before molecular analysis. The specimen was in turn deposited in the ichthyological collection of the Laboratório de Ictiologia, Ecologia Aquática, Universidade Federal do Pará, Belém, Brazil (voucher number GEA.ICT 02458). The measurements (to the nearest 0.1 mm using digital calipers) and meristic counts were taken on right side of the specimen.

Due to the overall similarity among the species of mangrove killifishes, the identification of the specimen was confirmed with molecular data. The total genomic DNA was isolated using the protocol proposed by DNA Kit Wizard Genomic Purification (Promega Corporation, Madison, Wisconsin, USA). A polymerase chain reaction (PCR) was conducted to obtain 450 bp of the mitochondrial gene 16S of the new recorded specimen (Genbank acession number KU987428) using primers from Palumbi (1996): forward 16SL1987 - 5′GCCTCGCCTGTTTACCAAAAAC3′; and reverse 16SH2609 - 5′CCGGTCTGAACTCAGATCACGT3′. The amplification was made in a final volume of 25 µL, containing 4 µl of DNTP (1.25 mM), 2.5 µl of buffer solution (10×), 1 µl of $MgCl_2$ (25 Mm), 0.25 µl of each initiator (200 ng/µl), 1–1.5 µl of genomic DNA (100 ng/l µ1), 1U of Taq DNA Polymerase, 1U (5U/µl), and purified water.

Figure 3. Habitat of *Kryptolebias hermaphroditus* at Ajuruteua beach, Brazil.

PCR cycling condition for amplification of 16S gene was: an initial denaturing step at 95 °C for 5 min; 35 cycles of denaturing at 94 °C for 40 s, annealing at 55 °C for 35 s, and extending at 72 °C for 1 min; and a final extending step of 72 °C for 5 min.

The Blast tool (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to test the genetic similarity to *Kryptolebias* species in order to molecularly identify the specimen. The obtained consensus sequence (Genbank acession number KU987428) was aligned to sequences of other rivulid killifishes from the South Atlantic coast, which have been made available by Murphy et al. (1999) using ClustalW (Thompson et al. 1994), implemented in the Bioedit 7.0.9 (Hall 1999). A neighbor-joining analysis was conducted through MEGA 6.0 (Tamura et al. 2013) to calculate the distances between species with the Kimura 2-parameter model (K2P) (Kimura 1980) and to test the molecular divergence among species.

The three *Kryptolebias* species (*K. hermaphroditus*, *K. marmoratus,* and *K. ocellatus*) have a very similar morphology, sharing a small flat head, terminal mouth and an elongate and cylindrical body with the anal-fin origin located before the dorsal-fin origin (Costa 2011, 2016). The body coloration, however, varies among species. In *Kryptolebias hermaphroditus* and *K. ocellatus* the dorsal profile is greenish brown, but in *K. marmoratus* it is pale brown. Additionally, the distinction among these species is mainly based on the color pattern of hermaphroditic individuals. The specimen of *K. hermaphroditus* from Ajuruteua beach (Figure 2) was identified based on the presence of a humeral spot slightly larger than the orbit and the caudal spot (vs. humeral spot approximately equal in size to caudal spot, and smaller than the orbit in *K. marmoratus*). The morphometric and meristic data for the voucher specimen of *K. hermaphroditus* are shown in Table 1.

The neighbor-joining topology (Figure 4) recovered a cluster with the specimen collected on Ajuruteua beach plus *Kryptolebias hermaphroditus* (sequence obtained from

Table 1. Morphometric and meristic data of the examined specimen of *K. hermaphroditus* from Ajuruteua beach, and for comparative data for this species from Costa, 2006, and 2011. Morphometric data are expressed as percentage of standard length, except when noted as percentage of head length. *n* = number of specimens included in the range.

Character	This study	Costa (2006) (*n* = 10)	Costa (2011) (*n* = 16)
% Standard length (mm)		Range	
Standard length	34.2	27.2–48.3	26.9–49.4
Head length	18.12	24.0–26.0	24.0–26.8
Body depth	18.71	17.5–19.7	17.5–21.6
Head depth	61.4	62.8–72.1	62.8–72.1
Caudal peduncle depth	13.16	12.3–13.4	12.3–14.2
Predorsal length	72.22	73.5–77.2	73.5–77.2
Dorsal fin base length	8.48	9.2–10.7	9.2–11.1
Anal fin base length	14.04	14.1–15.6	13.3–16.4
Pectoral fin length	13.16	17.4–19.0	17.4–19.8
% head length (mm)			
Eye diameter	30.65	28.4–33.0	27.8–33.0
Snout length	11.29	13.5-15.7	12.8–16.1
Counts (total)			
Dorsal-fin rays	8	8–9	8–9
Anal-fin rays	13	11–12	11–12
Pectoral-fin rays	13	13	13
Pelvic-fin rays	7	6	6

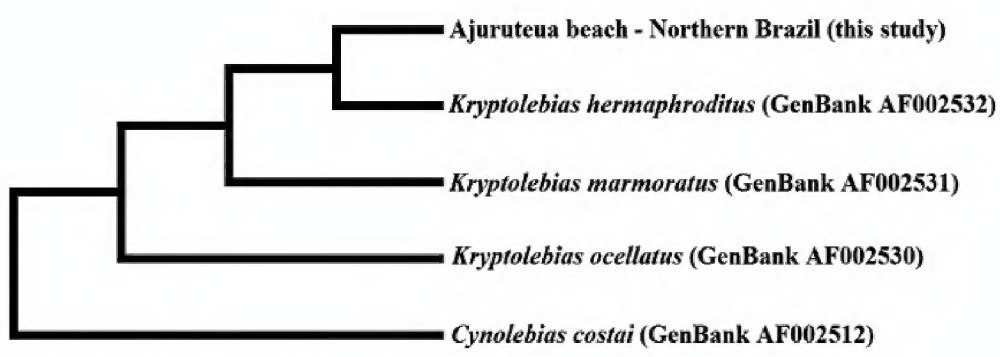
Ajuruteua beach - Northern Brazil (this study)
Kryptolebias hermaphroditus (GenBank AF002532)
Kryptolebias marmoratus (GenBank AF002531)
Kryptolebias ocellatus (GenBank AF002530)
Cynolebias costai (GenBank AF002512)

Figure 4. Neighbor-Joining phenogram based on 16S sequences showing the clustering between the specimen collected on Ajuruteua beach, some other *Kryptolebias* spp. and *Cynolebias costai*.

GenBank, accession number AF002532). The genetic distance between these two specimens was 0.7%. The voucher specimen also showed a greater genetic distance value compared to the congeners *K. marmoratus* (1.6%) and *K. ocellatus* (2.5%).

The large stretch of coast between Florida and the Caribbean region in the north and southeastern Brazil in the south presents a distributional gap with no records of any of the three *Kryptolebias* species (*K. marmoratus, K. ocellatus,* and *K. hermaphroditus*). Tatarenkov et al. (2011) questioned whether the absence of these species from other parts of the Brazilian coast could be attributed to a lack of sampling or whether this gap represents a real non-occurrence of these species. The new and recent records of *K. hermaphroditus* from Pará state (herein) and Rio Grande do Norte State (Lira et al. 2015) suggest that the real range of this species is underestimated due to a lack of intensive exploration in this region. Therefore, our record of *K. hermaphroditus* in Pará state is a valuable in revealing a notable distribution extension for this species along western Atlantic coast. Additional fieldwork sampling rivulid killifishes in the gap along the northeast coast of Brazil is recommended.

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